PUBLIC HEALTH LABORATORY SPECIMENS.

THEIR PREPARATION AND SHIPMENT.

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This paper has been prepared for the purpose of furnishing brief, concise instructions relative to the preparation and shipment of specimens for laboratory examination in order that the best results may be obtained.

Postal Regulations.

The shipping, through the United States mails, of diseased tissues, cultures, and other material capable of causing disease must be done in accordance with the provisions of the Postal Regulations, issued by the Post Office Department. Sections 472 and 473, Postal Regulations, 1913, read as follows:

SEC. 472. All kinds of poison, and all articles and compositions containing poison, and all poisonous animals, insects, and reptiles, and explosives of all kinds, and inflammable materials, and infernal machines, and mechanical, chemical, or other devices or compositions which may ignite or explode, and all disease germs or scabs, and all other natural or artificial articles, compositions, or materials of whatever kind which may kill, or in anywise hurt, harm, or injure another, or damage, deface, or otherwise injure the mails or other property, whether sealed as first-class matter or not, are hereby declared to be nonmailable matter, and shall not be conveyed in the mails or delivered from any post office or station thereof, nor by any letter carrier; but the Postmaster General may permit the transmission in the mails, under such rules and regulations as he shall prescribe as to preparation and packing, of any articles hereinbefore described which are not outwardly or of their own force dangerous or injurious to life, health, or property: *Provided*, That all spirituous, vinous, malted, fermented, or other intoxicating liquors of any kind are hereby declared to be nonmailable and shall not be deposited in or carried through the mails. Whoever shall knowingly deposit or cause to be deposited for mailing or delivery, or shall knowingly cause to be delivered by mail according to the direction thereon, or at any place at which it is directed to be delivered by the person to whom it is addressed, anything declared by this section to be nonmailable, unless in accordance with the rules and regulations hereby authorized to be prescribed by the Postmaster General, shall be fined not more than one thousand dollars, or imprisoned not more than two years, or both; and whoever shall knowingly deposit or cause to be deposited for mailing or delivery, or shall knowingly cause to be delivered by mail according to the direction thereon, or at any place to which it is directed to be delivered by the person to whom it is addressed, anything declared by this section to be nonmailable, whether transmitted in accordance with the rules and regulations authorized to be prescribed by the Postmaster General or not, with the design, intent, or purpose to kill, or in anywise hurt, harm, or injure another, or damage, deface, or otherwise injure the mails or other property, shall be fined not more than five thousand dollars, or imprisoned not more than ten years, or both.

2. Spirituous, vinous, malted, fermented, or other intoxicating liquors of any kind, poisons of every kind, and articles and compositions containing poison (except as prescribed in the fourth paragraph hereof), and poisonous animals, insects, and reptiles, and explosives of every kind, and inflammable materials (including matches, gasoline, naphtha, benzine, denatured alcohol, and all liquids having a flash point at or below 80° F.), and infernal machines, and mechanical, chemical, or other devices or compositions which may ignite or explode, and disease germs or scabs (except as prescribed in sec. 473), and other natural or artificial articles, compositions, or mate-

rials of whatever kind which may kill, or in anywise hurt, harm, or injure another, or damage, deface, or otherwise injure the mail or other property, live animals (except as prescribed in sec. 476), raw hides or pelts, guano, or any article exhaling bad odor, whether sealed as first-class matter or not, shall not be admitted to the mails.

3. Liquids not spirituous, vinous, malted, fermented, or otherwise intoxicating (including samples of altar or communion wine used in church services), and not liable to explosion or spontaneous combustion or ignition by shock or jar, and not inflammable, fruits or vegetable matter liable to decomposition, comb honey, soft soap, pastes or confections, ointments, salves, and articles of similar consistency, shall be admitted to the mails for transmission in the domestic mails when inclosed in packages in conformity with the conditions prescribed in sections 474 and 475.

4. Medicines and anesthetic agents which are not outwardly or of their own force dangerous or injurious to life, health, or property, and not in themselves unmailable (see secs. 454 and 480), may be admitted to the mails for transmission in the domestic mails when inclosed in packages in conformity with the conditions prescribed in section 474: *Provided*, That the terms "medicines" and "anesthetic agents" shall not be construed to mean poisons: *Provided further*, That the article mailed bears the label or superscription of the manufacturer thereof, or dealer therein, or of the licensed physician, surgeon, dentist, or veterinarian preparing or prescribing the same.

SEC. 473. Specimens of diseased tissues may be admitted to the mail for transmission to United States, State, municipal, or other laboratories in possession of permits referred to in paragraph 3 of this section only when inclosed in mailing cases constructed in accordance with this regulation: *Provided*, That bacteriologic or pathologic specimens of plague and cholera shall under no circumstances be admitted to the mails.

2. Liquid cultures, or cultures of microorganisms in media that are fluid at the ordinary temperature (below 45° C. or 113° F.), are unmailable. Such specimens may be sent in media that remain solid at ordinary temperature.

3. No package containing diseased tissue shall be delivered to any representative of any of said laboratories until a permit shall have first been issued by the Postmaster General, certifying that said institution has been found to be entitled, in accordance with the requirements of this regulation, to receive such specimens.

4. (*a*) Specimens of tubercular sputum (whether disinfected with carbolic acid or not disinfected) shall be transmitted in a solid glass vial with a mouth not less than 1 inch in diameter and capacity of not more than 2 ounces, closed by a cork stopper or by a metallic screw top protected by a rubber or felt washer. Specimens of diphtheria, typhoid, or other infectious or communicable diseases or diseased tissues shall be placed in a test tube made of tough glass, not over three-fourths of an inch in diameter and not over 7½ inches in length, closed with a stopper of rubber or cotton and sealed with paraffin or covered with a tightly fitting rubber cap.

(*b*) The glass vial or test tube shall then be placed in a cylindrical tin box, with soldered joints, closed by a metal screw cover with a rubber or felt washer. The vial or test tube in this tin box shall be completely and evenly surrounded by absorbent cotton, closely packed.

(*c*) The tin box, with its contents, must then be inclosed in a closely fitting metal, wooden, or papier-mâché block or tube, at least three-sixteenths of an inch thick in its thinnest part, of sufficient strength to resist rough handling and support the weight of the mails piled in bags. This last tube shall be tightly closed with a screw-top cover with sufficient screw threads to require at least one and one-half full turns before it will come off, and fitted with a felt or rubber washer (See fig. 8.)

5. Specimens of blood dried on glass microscopic slides for the diagnosis of malaria or typhoid fever by the Widal test may be sent in any strong mailing case which is not liable to breakage or loss of the specimen in transit.

6. Upon the outside of every package of diseased tissues admitted to the mails shall be written or printed the words "Specimen for bacteriological examination. This package to be pouched with letter mail."

Letters of transmittal.—It is advised that a duplicate letter of transmittal, giving data for each specimen, be written, one copy to be mailed to the laboratory as a letter, the other inclosed with the specimen. This is to guard against delay caused by loss of specimens or letters in transit. If both letter and specimen arrive at the same time, nothing is lost; if the specimen arrives before the letter, the necessary data are at hand so that examination can be proceeded with at once; if the specimen is lost and the letter arrives, after waiting a reasonable time, the laboratory can notify the sender and a second specimen may be forwarded.

The use of the form letter (see p. 2019) is recommended to save time and labor necessary in preparation of letters.

Reports.—In case report is desired by telegraph, the sender should request that he be so notified.

Interpretation of result.—In general, it may be stated that positive results obtained by laboratory methods are indicative of the disease or conditions found. On the other hand, negative results do not necessarily mean the absence of the disease or condition suspected. A single negative result is of relatively little value. The examination of several specimens with constantly negative results is of some value—the presumption of the absence of the disease growing stronger after each examination which gives a negative finding. In case of a negative result, the physician should feel no hesitancy in forwarding a second specimen for the same test, provided the case continues to show symptoms warranting the original suspicion.

Labeling, packing, and shipping, and data to be forwarded with specimens.

Labeling.—Each specimen should be labeled or marked as soon as prepared, so that no possible confusion of specimens can arise later. Specimens may be labeled by use of a gummed label (written preferably with graphite pencil), by writing on container or slide with a wax pencil, or with a point for writing on glass. The label should state the source of the specimen and the character of examination desired. Special considerations in labeling will be taken up when discussing the class of specimens requiring them.

Packing.—Specimens of blood on slides should be packed so that the specimen will not be subject to friction. Postal regulations do not prescribe any restrictions for this class of specimen except that they shall be packed in such manner as to insure their safety against breakage en route.

Blood serum in sealed pipettes or in sterile vials, and feces, urine, sputum, and tissues placed in bottles as specified in this pamphlet should be wrapped well in cotton to insure against breakage and placed in a double mailing case as specified by postal regulations.

Water specimens usually require packing in ice. Special containers for this purpose may be had, but one may be improvised by placing the specimen bottles in a water-tight wooden container (wooden buckets or butter tubs are suitable), surrounding it by ice, and placing sawdust over the ice.

Cultures must be on media which is solid at ordinary temperatures and in test tubes of tough glass closed by a paraffined stopper or rubber cap. Gelatin cultures are unmailable, as these liquefy at ordinary temperatures. Cultures of plague and cholera are unmailable.

Shipping.—Nearly all specimens can be sent by mail, though specimens which have to be shipped on ice must be sent by express. All materials capable of transmitting cholera or plague are unmailable and must be sent by express. All specimens which are placed in alcohol must be sent by express, as alcohol is unmailable.

Data to accompany each specimen.—These data are set forth in the form letter shown below.

....
(Station.) (Date.)

DIRECTOR, ... LABORATORY.

.....

SIR: The specimens indicated below are being forwarded to you to-day by.....
..... (Mail or express.)

Number of specimens.	Nature of specimen.	Marks of each specimen.	Check space.	To be examined for—
.....	Blood smears.....	.....	.....	Differential blood count.
		.....	.....	Malarial parasites.
		.....	.....	Agglutination against..... (State organism.)
.....	Blood, dried.....	.....	.....	Other tests.
		.....	.....	Agglutination against.....
.....	Blood, serum.....	.....	.....	Wassermann.
		.....	.....	Complement fixation for..... (State disease.)
.....	Blood culture.....	.....	.....	..... (State organism suspected.)
.....	Cultures on..... (State medium.)	.....	.....	Diphtheria.
		.....	.....	Preparation of a vaccine.
		.....	.....	Identification.
		.....	.....	B. typhosus.
		.....	.....	Other organisms..... (State organisms.)
.....	Feces.....	.....	.....	Animal parasites or eggs..... (State parasite.)
		.....	.....	Tubercle bacillus.
.....	Sputum.....	.....	.....	Other organisms..... (State organism.)
		.....	.....	Chemical examination.
.....	Urine.....	.....	.....	Typhoid bacillus.
		.....	.....	Other organisms..... (State organism.)
		.....	.....	Bacterial count.
.....	Water.....	.....	.....	Colon determination.
		.....	.....	Chemical examination.
.....	Pathologic tissue for examination.	.....	.....	Malignancy.
		.....	.....	Other conditions.....
	Fixed in..... (State reagent.)	.....	.....	(State special conditions for which examination is desired.)
	Shipped in..... (State fluid.)	.....	.....	
.....	Zoological specimens.....	.....	.....	For determination.
.....	..... (State host and locality.)	.....	.....	

DATA DESIRED FOR EACH SPECIMEN.

Specimen marked ..... Collected on ..... (Date.)

Source.....

If from patient..... ..... (Name or No.) (Age.)

.... (Race.) (Sex.) (Occupation.)

Duration of illness.....

Quinine administered..... (For malaria suspects only.) (Date of last dose.)

Tissues removed—ante mortem or post mortem.....

Organ or portion of body from which tissues were taken.....

Duration of growth..... } In case of new growths only.

Previous removal, if any ..... } (Date.)

Remarks.....

The following list contains practically all the classes of specimens that are submitted for laboratory examinations:

1. Blood:
   - Smears, thin.
   - Smears, thick.
   - Dried blood for agglutination test.
   - Fluid blood for agglutination test.
   - Serum for agglutination test.
   - Serum for complement fixation tests.
   - Cultures for *B. typhosus* or other organisms.
2. Cultures (pure and mixed):
   - Suspected diphtheria cultures.
   - Mixed cultures from which vaccines are to be made.
   - Cultures of organisms for identification.
3. Feces:
   - For bacteriological examination.
   - For zoological examination (for animal parasites or their ova).
4. Sputum.
5. Urine:
   - For bacteriological examination.
   - For chemical examination.
6. Water:
   - For bacteriological examination.
   - For chemical examination.
7. Pathological tissues for sections.
8. Entomological specimens for determination.
9. Helminthological specimens for determination.
10. Miscellaneous.

Each class of specimens will be considered with reference to the following general subdivisions:

A. Conditions in which the examination may give the desired result.

B. Technique of obtaining the specimen.

C. Special precautions to be observed and reasons for same.

1. Blood Specimens.

(*a*) Thin smears of blood are used in examining for the presence of blood parasites (malaria, filariasis, trypanosomiasis, etc.), for differential leucocytic count, and examination for the anemias or leukemias.

(*b*) Such smears should be made on clean glass slides. These slides should be cleansed with soap and water, followed by immersion in acid alcohol (HC1 3, alcohol 97) for several hours. If the slides are new it is advisable to immerse them in strong sulphuric acid overnight and rinse them thoroughly before placing in acid alcohol. It is convenient to keep the slides stored in acid alcohol, wiping them with a piece of clean gauze or tissue paper just prior to using. In handling the slides care should be taken to touch only the edges, as touching the flat surface leaves a thin film of grease from the skin upon the glass. This will interfere with the uniform spreading of the blood film. Forceps for handling the slides are an aid in keeping the glass clean.

The specimen is obtained from the finger or the lobe of the ear in the usual manner. The skin should be sponged thoroughly with alcohol, followed by ether to remove grease from the surface. A sterile needle or lancet should be used to make the puncture. The blood should flow freely and hastening the flow by compression of the parts should be avoided, especially if an examination for evidence of the leukemias or anemias is desired. The first drop of blood should be wiped away. A large drop of blood is not desirable as it will make the film too thick. A drop the size of a pinhead, or a No. 6 bird shot, is sufficient for one smear. The drop of blood is touched by the flat surface of the slide, near one end, the slide then placed in such position that the drop is on the upper surface, and the edge of a second slide, held at an angle of 30 to 45 degrees, is placed in the drop and pushed toward the other end of the first slide (see fig. 1, A and B). In this way the corpuscles are spread by capillary action and not distorted by pressure between the edge of the spreading slide and the surface of the specimen slide. The movements used in spreading the blood should be executed fairly rapidly with a steady hand to insure uniform distribution.

Thin smears may also be made by touching the drop of blood on the skin of the finger or ear with the edge of the spreading slide and then smearing the other slide with it.

Allow the smears to dry without heating.

Several slides should be prepared from each case as some may be failures, or it may be desirable to employ more than one stain in the examination.

(c) *Cautions.*—As the examination for the different types of white corpuscles and changes in all cellular elements of the blood is made from thin smears, it is important that the blood taken be as nearly representative of the case as is possible. On this account, compression of the tissues to facilitate bleeding is to be avoided, as such procedure may increase or decrease the various elements of the blood. For example, squeezing the tissues may cause the exudation of lymph from the tissues. This is poor in red cells but rich in white cells. The lymph is added to the blood, thereby decreasing the relative number of red cells and increasing the relative number of white cells. This is especially important if a differential count is to be made.

The thin film is also necessary to determine the species of malarial parasites.

(a) The thick blood smear is used only in examination for the presence of malarial, or other, parasites. It need not be sent unless examination for parasites is desired. . Its object is to enable the microscopist to examine a large amount of blood in a shorter time. In thick smears only the presence of parasites can be determined; if determination of species is desired, thin smears must be sent.

(b) The following technique of Von Ezdorf is recommended for making the thick blood smears:

Obtain a large drop, or two, of blood on the surface of a glass slide 1 inch from the end.

With the convex surface of a steel writing pen stuck in a cork, the blood is spread evenly over an area about one-half to three-fourths inch in diameter. The pen should be wiped clean immediately after using.

A second thick smear is made on the slide at the other end (see fig. 2).

(c) *Cautions.*—It is desirable to get specimens for malarial examination before quinine has been administered, as the use of this remedy may make the finding of parasites more difficult or uncertain.

Fixation of blood smears need not be done, as the dried smear, if properly packed for shipment, will reach the laboratory in good condition. Heat should not be used on blood smears as it will ruin the specimen for staining with the better blood stains. The method of labeling of Von Ezdorf, writing on the edge of the blood smear with a soft lead pencil, may be employed.

Blood for Agglutination (Widal) Test.

(a) This test is applicable to cases of typhoid fever, paratyphoid fever, bacillary dysentery, Malta fever, and cholera.

There are two methods of obtaining blood for this test, the dried blood and fluid blood (serum) methods. The latter is much preferable as it is more accurate and more certain of results.

(*b*) *Technique.*—In the dried blood method the blood is obtained by pricking the finger or lobe of the ear and depositing several large drops of blood on a clean glass slide; the drops are allowed to dry without spreading. Five separate drops can be placed on one slide without difficulty (see fig. 3).

(*c*) *Cautions.*—Do not use dirty, greasy, or rough glass; do not collect specimens on tin, zinc, or other metal as the blood may not adhere to the metal when perfectly dry. Such specimens are frequently jarred loose from the metal and lost in the wrappings upon opening.

(*b*) For fluid specimens for agglutination tests the serum is all that is required. The best results are obtained when the blood is drawn from a vein with a syringe, placed in a sterile test tube, allowed to clot, and the serum removed from the clot with a sterile pipette. The technique of this procedure is given fully under collection of specimens for complement fixation tests. Five cubic centimeters of blood will be sufficient for the agglutination test.

Fluid specimens may be collected in capillary pipettes and shipped without separating the serum from the clot. This procedure is not as desirable as the syringe method but gives better results than the dried-blood method (see fig. 4).

(*c*) *Caution.*—In sealing capillary tubes care must be taken not to heat the blood, as this may destroy the agglutinating properties of the serum, rendering the specimen worthless. To guard against this, hold the tube with the fingers placed at the level of the blood column nearest the portion of the tube to which heat is being applied.

Blood Specimens for Complement Fixation Test (Wassermann).

(*a*) This test is at present applied chiefly in cases of suspected or confirmed syphilis, though it is applicable in principle to certain other diseases, such as gonorrhea, tuberculosis, and other infectious diseases.

For this test a specimen of clear serum is essential for obtaining good results. Sera which are discolored by laking of the red corpuscles may not give a definite result, and the reading of the hemolysis may be interfered with if hemoglobin be present in the serum; on this account the serum should be separated from the clot before shipment.

(*b*) *Technique.*—The following procedure is recommended: Ten cubic centimeters of blood should be drawn from a vein with a sterile needle and syringe, under aseptic conditions. Place the blood in a sterile test tube or a centrifuge tube, if a centrifuge be available.

The tubes should be stoppered by a sterile cotton plug or a sterile cork. After the clot becomes firm separate it from the wall of the tube with a sterile needle, and then set the tube in a cool place to

allow the clot to contract. Placing it in an ice box until the next morning is sufficient. If a centrifuge be available, a clearer specimen is obtained by centrifugalizing; if not, a satisfactory specimen can be obtained by removing the clear serum with a pipette, or carefully decanting it into a sterile rubber-stoppered glass vial. The stopper should be firmly inserted and, as a further precaution, a strip of adhesive plaster passed across the top of the stopper and down the sides of the vial (see fig. 5).

(*c*) *Cautions.*—The following points are necessary to obtain good results. Blood should be drawn before meals to avoid an excess of chyle in the serum. Specimens for the Wassermann test should not be taken after the ingestion of alcoholic beverages, as this interferes with the test.

As specimens of this nature may be taken from more than one person at one time, it is necessary that each tube be marked or labeled so that confusion of specimens may not arise later. To guard against this, the labeling should be done as soon as a specimen is placed in the vial.

Blood Cultures.

(*a*) The use of blood cultures in the diagnosis of typhoid fever furnishes us a method of positive diagnosis prior to the appearance of the Widal reaction in many cases. When positive, this result is absolutely certain, as the *Bacillus typhosus* is isolated and identified. Blood cultures should be employed whenever possible, as the results are more satisfactory and obtained at an earlier date than when the agglutination test is relied upon. The blood culture should be made early in the disease, as the following results of Buxton and Coleman (Am. J. M. Sc., vol. 133, p. 896) show:

Cases examined in first week of disease gave 89 per cent positive.
Cases examined in second week of disease gave 73 per cent positive.
Cases examined in third week of disease gave 60 per cent positive.
Cases examined in fourth week of disease gave 38 per cent positive.
Cases examined after fourth week of disease gave 26 per cent positive.

For typhoid blood cultures a special medium of ox bile is preferable, though ordinary bouillon may be used. About 20 cubic centimeters of bile should be placed in a bottle of 30 to 50 cubic centimeters capacity, fitted with a ground-glass stopper. The bottles, with their contents, are then sterilized in streaming steam for one and one-half hours.

(*b*) *Technique of making culture.*—To obtain blood use a sterile needle and syringe as in collecting specimens for Wassermann test; 10 cubic centimeters of blood should be drawn and at once placed in two bottles of media, 5 cubic centimeters to each bottle. The stopper is then replaced and held in place by a strip of adhesive plaster. The large amount of blood is used to insure obtaining the

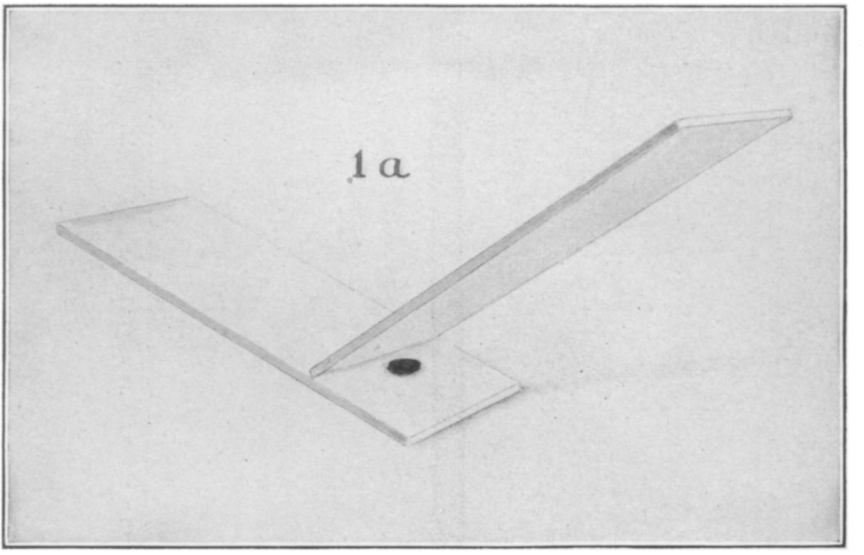

FIG. 1a.—DROP OF BLOOD JUST PRIOR TO APPLICATION OF SPREADING SLIDE.

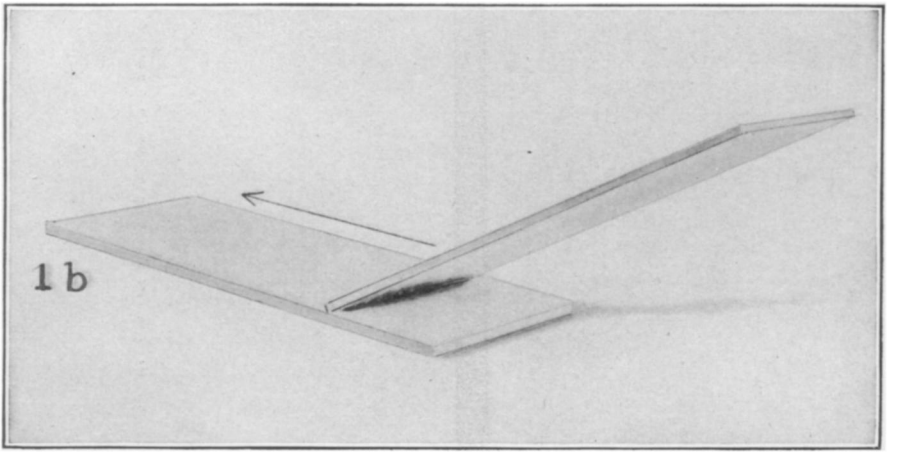

FIG. 1b.—METHOD OF SPREADING THE SMEAR.

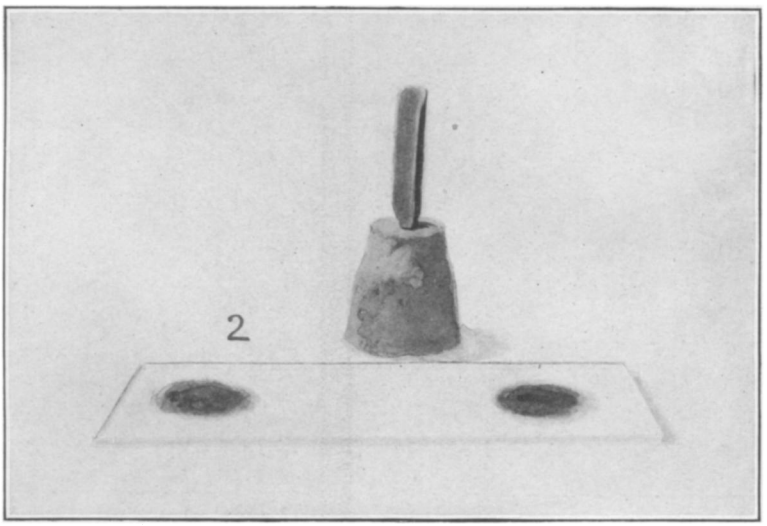

FIG. 2.—THICK SMEAR OF BLOOD AND PEN USED FOR SPREADING SAME.

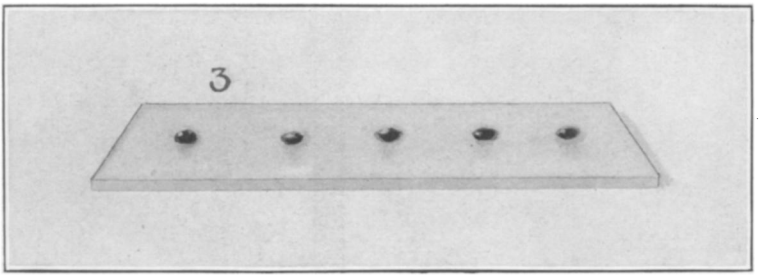

FIG. 3.—SPECIMEN OF DRIED BLOOD FOR AGGLUTINATION TEST.

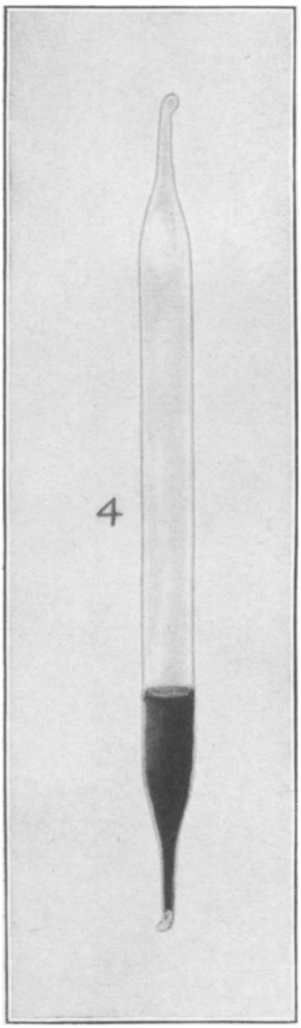

FIG. 4.—SPECIMEN OF BLOOD IN SEALED CAPILLARY TUBE.

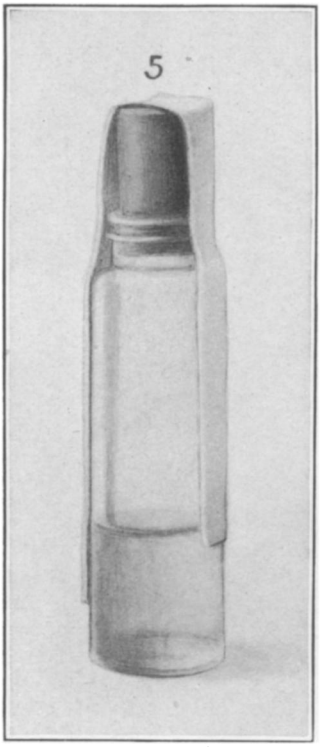

FIG. 5.—SPECIMEN OF BLOOD SERUM FOR WASSERMANN TEST.

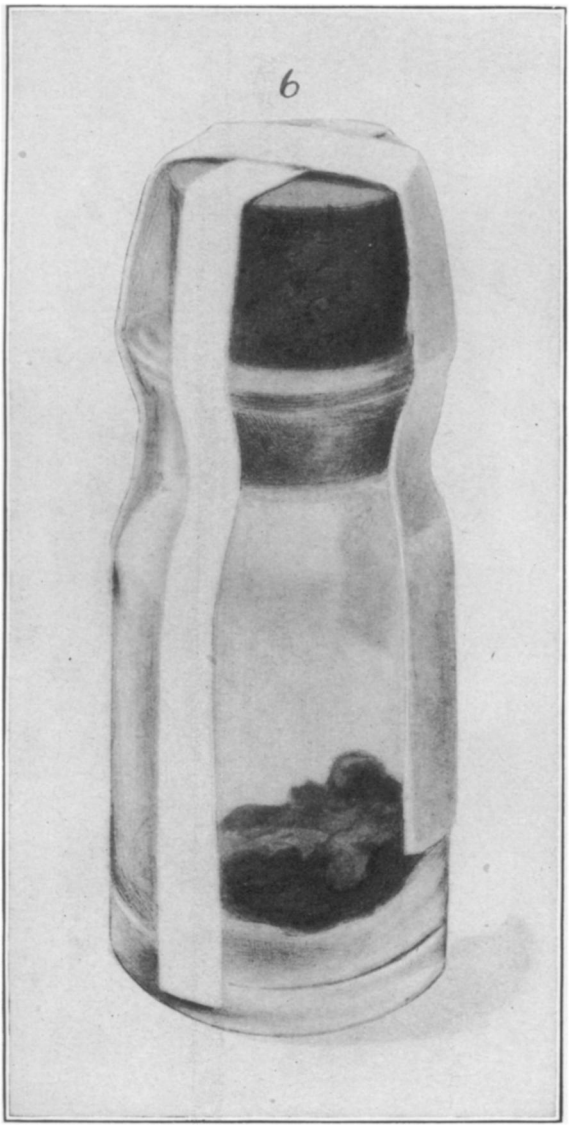

FIG. 6.—SPECIMEN OF FECES PACKED FOR MAILING.

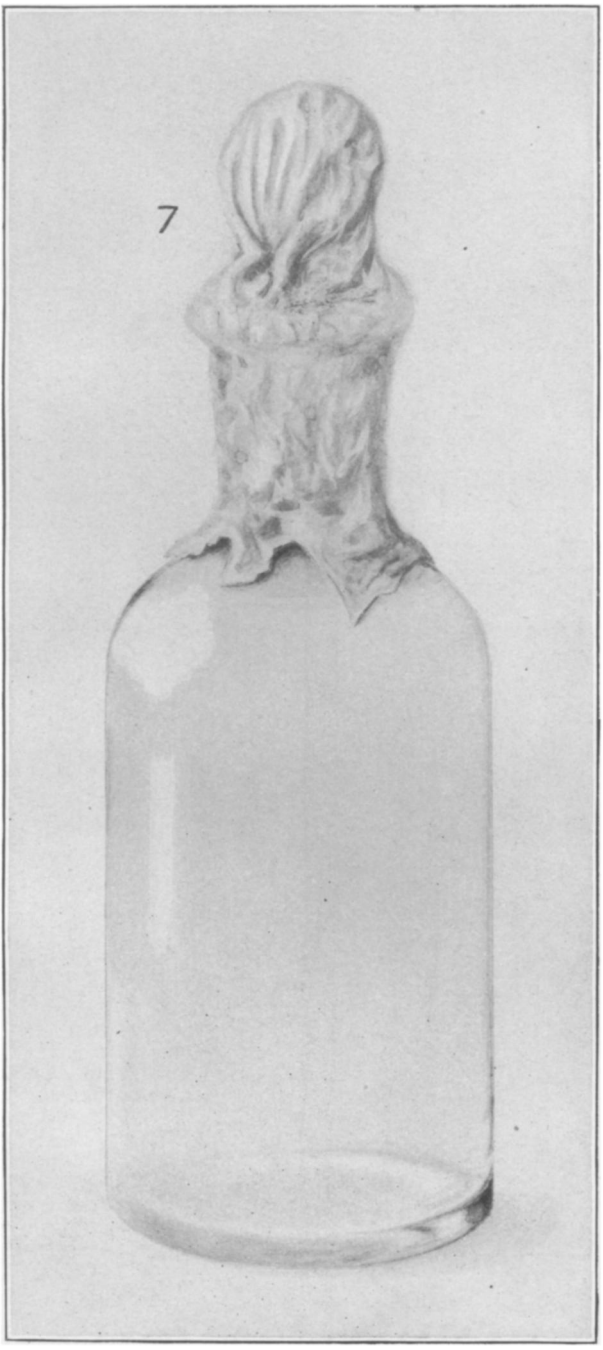

FIG. 7.—WATER SAMPLE BOTTLE FOR BACTERIOLOGICAL SPECIMEN.

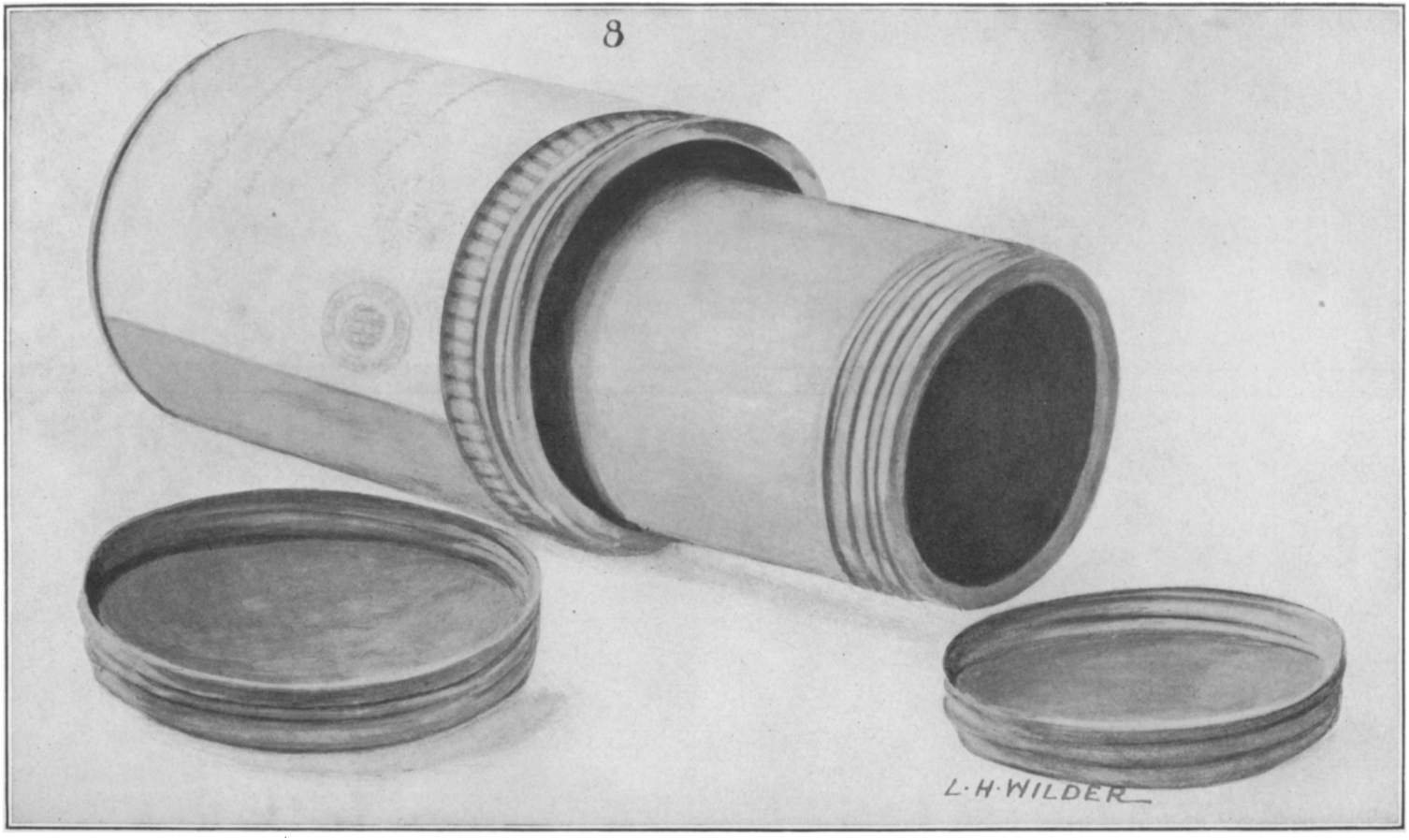

FIG. 8.—MAILING CASE.

organism sought, if present. This procedure is also applicable in other bacteriemias.

(*c*) *Caution.*—As the shipment of cultures in fluid medium is prohibited by postal regulations, these cultures should be shipped by express. They should be wrapped in sufficient cotton to insure absorption of all fluid in case the containers are broken, and then placed in a box or other container that will guard against breakage.

2. Cultures, Pure and Mixed.

(*a*) This class of specimens is used chiefly in suspected cases of diphtheria but is also applicable to other diseases, particularly the sending of a mixed culture from pus or other exudate, from which the preparation of autogenous vaccines is desired.

(*b*) *Technique.*—A sterile swab should be used to obtain the specimen from the nose, throat, or lesion. These swabs should be prepared and sterilized in test tubes. The swab is removed from its tube and rubbed over the lesion, then rubbed over the surface of the medium, and then replaced in its original tube. The inoculated tube of medium and the tube containing the used swab should both be forwarded.

(*c*) *Caution.*—Though most cultures taken in this way are mixed cultures, it is essential that all extraneous organisms be avoided, as many, especially the molds, will rapidly overgrow the pathogenic organisms and prevent their development.

For use in diphtheria Löffler's blood serum is the preferable medium. For pus, or vaccine specimens, agar-agar or serum may be used.

In case pure cultures are sent from one laboratory to another for identification or confirmation, complete data concerning the culture should be inclosed.

3. Feces.

(*a*) Feces are usually submitted for examination for evidence of parasites, or to determine the presence of certain organisms, particularly the typhoid bacillus. The preparation of the specimen is the same for both bacteriological and zoological examination.

(*b*) *Technique of preparation of specimens.*—A wide-mouthed bottle having a capacity of about 50 cubic centimeters is the best container. A well-fitting rubber or cork stopper is necessary. The bottle should be filled to about one-third of its capacity with the feces to be examined, the stopper inserted firmly and held in place by a strip of adhesive plaster passed across the stopper and down the sides of the bottle (see fig. 6).

The use of 20 per cent of glycerin in physiological salt solution has been reported by Cumming (Jour. of Amer. Med. Assn., vol. 68,

p. 1163) to have an inhibitory effect on the colon bacillus and other fecal organisms.

He recommends the use of the following solution:

Physiological salt solution (0.9 per cent NaCl)	80
Glycerin	20

To 25 cubic centimeters, add a mass of feces about the size of a split pea, and shake well to make a uniform suspension. Do not use a greater amount of feces, as the inhibition of *B. coli* may not be obtained when large amounts are used.

This method gives admirable results on specimens artificially contaminated with laboratory strains of *B. typhosus* and should be applicable in practice.

(*c*) *Cautions.*—Frequently specimens of feces are received for bacteriological examination that have been subjected to the action of disinfectants, which renders them useless for cultural studies. This is probably the result of a misunderstanding on the part of the attendant, or, perhaps, the patient.

The isolation of the typhoid bacillus from stools becomes more difficult in direct proportion to the age of the specimen. For this reason it is essential that the specimen be forwarded to the laboratory without delay. This also has a bearing on the interpretation of results of the examination, as a specimen that is in transit for a long period may give negative results, although the same specimen at the time of collection would give positive findings.

It is also quite definitely proven that in known typhoid carriers the administration of a cathartic increases the number of typhoid bacilli in the stools. In view of this fact a purge should be given the suspect a few hours before the specimen is to be collected, and a sample of the soft or semifluid stool resulting therefrom forwarded for examination.

The bottle should not be filled to more than one-half, preferably one-third, of its capacity. The reason for this is that a certain amount of gas is formed as a result of fermentation. If a large amount of fecal material be present a larger amount of gas is liberated, and it may displace the cork, allowing the contents to escape and possibly causing disease in those handling the specimen. A small amount of feces gives less gas formation and the empty two-thirds of the container's capacity allows space for a small amount of compression of the gas.

The cork should be large enough to allow at least 1 centimeter (about one-half inch) of it to project above the mouth of the bottle. A cork that is pushed in until level with the mouth of the bottle is hard to remove with safety.

Feces of cholera patients or suspects are unmailable and must be sent by express.

4. Sputum.

(*a*) Sputum is usually forwarded for examination for the presence of tubercle bacilli, though at times, examination for other organisms, or for the ova of parasites, may be desired. As many of the pathogenic organisms in sputum, other than tubercle bacilli, are easily killed, the examination for such organisms may not be reliable, as the particular organism may have died out in transit. Under ordinary conditions the examination of sputum for tubercle bacilli can be done by the physician or an assistant. As it may be desired to submit specimens from doubtful cases, for confirmation of diagnosis, the following directions should be followed:

(*b*) *Preparation of specimen.*—The directions given above for the preparation of specimens of feces for shipment apply also for specimens of sputum, except that glycerin should not be used.

(*c*) *Caution.*—Specimens of sputum which have been disinfected by carbolic acid will show the presence of tubercle bacilli under the microscope. It is recommended, however, that specimens be sent without disinfectant, in order that animal inoculations may be made, if microscopic examination is negative.

5. Urine.

(*a*) Specimens of urine for chemical examination will usually decompose en route, so that this examination is not practicable unless the distance to the laboratory is very short. The ordinary chemical examination for albumen and sugar can be done by the physician. If it is desired that an examination for some substance not easily detected by ordinary methods be made, a specimen may be forwarded by adding a preservative, such as chloroform, or by icing and shipping by express.

(*b*) Urine for bacteriological examination, such as that of typhoid patients, should be placed in a small vial closed with a rubber stopper which is held in place by adhesive plaster. The vials (10 cubic centimeters) used for shipping serum for Wassermann tests are large enough.

(*c*) *Caution.*—No preservative should be added if a bacteriological examination is to be made. Urine of cholera patients or suspects is unmailable.

6. Water.

In general terms, the results obtained by examination of water at a distance from the source of the specimen are only approximate. Time is the important factor, and to obtain the most accurate results, arrangments for examination of the water at, or near, the place where samples are collected should be made.

(*b*) If a sanitary chemical examination is desired the sample should be at least 2 gallons, placed in chemically clean bottles, and should be not over 48 hours old when received at the laboratory. Examination for mineral content can be made from older samples.

Specimens for bacteriological examination should be placed in 50 or 100 cubic centimeter ground-glass stoppered bottles, which have been sterilized by heat, the stopper and necks of the bottles being protected by lead foil (see fig. 7). The collection of the samples requires much care to prevent the addition of extraneous organisms to the sample. If a sample is taken from a tap, let the water run several minutes, then remove the stopper from the sample bottle and hold under the tap until filled. Replace the stopper without touching the neck of the bottle or that part of the stopper which is to come in contact with the water. Replace the lead foil, if it has been removed from the stopper. It is preferable to unwrap the lead foil from the neck of the bottle and then remove the stopper in its foil covering.

If a sample is to be taken from a pump, similar precautions should be taken. If from a stream, the mouth of the bottle is pointed up stream. In general terms, hold the mouth of the bottle so that water which accidentally touches the hands will not enter the container. Many specimens of water are probably grossly contaminated by carelessness in the taking of the sample. After obtaining the specimen it should be at once packed and shipped in a container with ice.

There are two kinds of bacteriological tests for water samples—an estimation of the number of bacteria per cubic centimeter and a test for the presence of the colon bacillus, which is presumptive evidence of pollution. If a bacterial count is to be made, the specimen should be examined as soon as possible after the sample is taken, and, at most, not later than 24 hours from time of collection. If a greater time be required in transit, the bacterial count may not be reliable, especially if the sample be exposed to temperatures much above 10° C.

For testing for evidence of pollution (colon bacillus test), samples may be as much as 48 hours old, but if the result is negative, it should not be interpreted as meaning an absence of pollution, as colon bacilli may have been present and died en route. In this, as in all laboratory tests, a positive finding is much more significant than a negative one. Repetition of the test for a number of times with consistently negative results may warrant the deduction of a negative finding, but a single negative result does not necessarily mean that the suspicion of contamination is not well founded.

(*c*) *Cautions.*—Most of the precautions have been touched upon above when considering collection of samples. It is necessary to

ship water samples on ice, and sufficient ice should be used to insure the arrival of the specimen at its destination before the ice is completely melted.

A gummed label should be attached to each sample stating its source and date of collection. To guard against loss of the label by reason of the moisture in the packing, place a rubber band around the bottle at the level of the label.

7. Pathologic Tissue for Section.

The preparation of sections of tissues, and the results obtained, depend largely on the fixing fluid used. There are several fluids that may be used, but some are much superior to others.

Zenker's fluid.—This is the most satisfactory fixing fluid for general use. Its formula is as follows:

Potassium bichromate	2 to 2.5 gm.
Corrosive sublimate	5 gm.
Water	100 cc.
Glacial acetic acid	5 gm.

The first two ingredients are dissolved in the water and the acetic acid is added just before using. (The original formula of Zenker's fluid contained sodium sulphate, but this has been found unnecessary.)

The tissues should be placed in this fluid 12 to 24 hours; then washed in running water 12 to 24 hours to remove the excess of fixing fluid. Then place the tissues in 80 per cent alcohol for shipment. Do not ship tissues in Zenker's fluid.

Formaldehyde solution.—A simple fixing fluid is 4 per cent formaldehyde solution, made by diluting one part of commercial (40 per cent) formaldehyde solution (formalin) with 9 parts of water. If great haste is desirable this is the fluid to be chosen, as it allows the making of frozen sections on receipt of the specimen. Furthermore, the tissue can be placed in it and mailed at once, fixation taking place en route. If haste is not necessary, the fixing action of formaldehyde is improved by adding 5 per cent of glacial acetic acid to the fluid and after 24 hours transferring to a 4 per cent formaldehyde solution (without acid) for shipment.

Alcohol.—Alcohol, though the least efficient, is probably the most popular of all fixing fluids, since it requires little or no preparation before using. It is especially valuable for specimens in which it is desired to determine the presence of bacteria. However, for purely histological sections, it does not give as good fixation as the other fluids and, as a result, poorer sections are obtained. Furthermore, if tissues containing skin are placed in alcohol, they become so hard before reaching the laboratory that sectioning is almost impossible. The use of alcohol as a fixing agent is not recommended, except when

the presence of bacteria is to be demonstrated, though if necessity requires, it should be used in 80 per cent strength for 2 to 4 hours, then in 95 per cent strength for 6 to 12 hours and then in 80 per cent for shipment.

(*b*) *Technique of preparation of specimens.*—On removal from the body of the tissues that are to be examined small blocks of tissue, not more than 5 millimeters in one dimension, should be cut with a sharp knife or razor from that portion of the specimen that seems most involved and dropped into the fixing fluid at once. If the specimen be small the whole specimen should be fixed in the chosen fluid; if large, place the small blocks in the chosen fixing fluid and the remainder in 4 per cent formaldehyde solution and forward the large portion of the specimen for macroscopic examination.

The attainment of fixation before cell death has begun is a most important point in the primary treatment of pathologic specimens. To secure this it is essential that delay in getting the specimens into fixing fluid be reduced to a minimum. In order to avoid delays it is suggested that autopsy rooms be stocked with a quantity of fixing fluids, glacial acetic acid, measuring graduates, etc. At autopsy the obtaining of the specimen is of primary importance. In case of specimens removed at operation the specimen, of course, is subordinate to the welfare of the patient. If, however, an assistant or interne can take the specimen as soon as removed and place it in fixing fluids it will be much better than allowing it to remain in the air until the operation is completed.

The small blocks of tissue, after being treated as outlined under the consideration of fixing fluids, should be placed in a wide-mouthed bottle in the proper medium, securely corked, and the cork held in place by a strip of adhesive plaster.

The following considerations relative to certain tissues should be observed in cutting small blocks for examination:

In case of new growths several blocks from various parts of the growth should be taken. At least one should be taken from the edge of the growth showing both normal and diseased tissue.

Blocks from the lung should be cut to include pleura and, if possible, a bronchial lymph gland.

Blocks from the kidney, liver, and spleen should be cut to include a portion of the capsule.

Blocks from the heart should be cut to include the base of a papillary muscle.

Muscle tissue should be preserved in 4 per cent formaldehyde as it is prone to become very hard in alcohol.

8. Entomological Specimens.

Mosquitoes.—Mosquitoes intended for shipping to a laboratory for identification should be killed with tobacco smoke or chloroform vapor and packed, while still soft, in pill boxes between two thin layers of cotton, packed loosely, just enough cotton being used to prevent shaking; in packing, the specimen should be handled as little as possible and very gently. Tin boxes and glass vials are to be avoided as the retained moisture causes the specimens to mold. Pill boxes, the covers of which can be lifted off, should be used as sliding covers usually ruin the specimen.

Collections made in moist climates must be well disinfected with creosote or some similar substance. The presence of a small amount of gum camphor or naphthalene in the container will preserve over great distances.

The pill boxes to be sent by mail should be securely packed in a strong outer box or mailing case that will not be crushed in transit.

Larvæ and pupæ are best preserved in 80 per cent alcohol.

Ticks and fleas can be killed and preserved by dropping them in 70 per cent alcohol.

9. Helminthological Specimens.

Helminthological specimens should be placed in 4 per cent formaldehyde solution or in 70 per cent alcohol to which 5 per cent of glycerin has been added and shipped in these fluids.

10. Miscellaneous.

Cerebrospinal, ascitic, hydrocele, or hydrothorax fluids should be collected in sterile glass-stoppered bottles, the stopper being protected by a sterile wrapping of lead foil, canvas, gauze, or paper. The chief precaution to be observed is that of aseptic technique, so that no extraneous infection may gain entrance.

The label should show the nature of the specimen, name of patient, and character of examination desired.

Small specimens may be mailed, large ones should be sent by express.

Complete data concerning the case are desirable.

As these fluids, especially hydrocele and ascitic fluids, when sterile, are of value for the preparation of special media, it is suggested that those taking specimens of such fluids collect them under aseptic conditions and forward them to the nearest laboratory. Antiseptic substances should not be added to the specimen, or used on instruments employed in obtaining the specimen.

Anthrax.—Tissues from cases of suspected anthrax in man or animals should be placed in a sterile glass container, securely stoppered, packed in cotton, and mailed in a double mailing case with a statement of the history of the case and disease suspected. Blood of animals dying of anthrax may also show the presence of the organism.

Rabies or hydrophobia.—The brains of animals suspected of rabies may show microscopic evidence of the disease.

(*b*) The head of the suspected animal should be severed from the body and the whole head packed in a suitable container and shipped by express. In warm weather it should be iced.

(*c*) *Caution.*—Heads should be sent as soon as possible after the animal dies or is killed. Killing the animals by chloroform or drowning is preferable to shooting or the application of violence about the head, as the latter is liable to lacerate the brain and facilitate the entrance of putrefactive organisms.

The name, post-office address, and telegraphic address of the sender should accompany the head, since an early report in these cases is important. If an examination other than for evidence of rabies is desired it should be stated; otherwise, on receipt of an animal's head, the presumption is that examination for rabies is desired. In case the brain is removed from the cranium and shipped in a container, the nature of the examination desired should be stated.

Plague.—It may be desirable to send dead rodents suspected of being infected with *Bacillus pestis* to a laboratory for examination. If the presence of plague among the rodents of a community has been proven, a plague laboratory should be established at a convenient point. In case plague is suspected but not proven, shipment to a laboratory may be done. Dead rodents should be placed in an insect-proof metal container as soon as captured and a small amount of chloroform poured into the container to kill fleas or other ectoparasites. Each rodent should have a tag attached to the carcass showing the locality where it was captured or found dead. The total collection of rodents for the day should be placed in a flea-proof metal container or can which can be sealed to prevent its being opened en route. This is shipped by express. The use of ice in the container is indispensable.

(*c*) *Caution.*—The protection of the collector against fleas is a point to always have foremost in the mind of those engaged in the work. The attachment of the tag is very important as on this depends the locating of the infected zone. Do not use too small a container or attempt to economize on ice.

The shipment by mail of plague cultures, or materials capable of transmitting plague, is prohibited.